Resistance to Picrotoxin Poisoning Induced by Catatoxic Steroids

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Abstract

In rats, severe and almost always fatal picrotoxin poisoning can be prevented by various steroids such as ethylestrenol, SC-11927, spironolactone, norbolethone, oxandrolone, prednisolone and progesterone. On the other hand, triamcinolone, desoxycorticosterone, hydroxydione, cholesterol and β-sitosterol do not prevent picrotoxin convulsions.

There appears to be a close relationship between the ability of various steroids to protect against picrotoxin convulsions and their catatoxic actions previously observed in other test systems.

It has long been known that adrenalectomy decreases resistance to most toxic agents whereas substitution therapy with corticoids restores it to normal. This protection is effected mainly by combating stress to which the organism is particularly sensitive in the absence of the adrenal cortex. Yet overdosage with corticoids is singularly ineffective in raising nonspecific resistance in intact animals above normal, presumably because a near optimal corticoid supply is assured by the physiologic activity of the adrenal cortex [1]. Only against a few agents, such as bacterial endotoxins [2] is it possible to raise resistance far above normal by treatment with glucocorticoids.

However, several reports from this laboratory have shown that certain steroids (not necessarily endowed with corticoid potency) can protect the intact rat against various types of severe intoxications. For example, these 'catatoxic steroids' (from the Greek kata = down, against) [3] can induce resistance against steroid anesthesia [4], pentylenetetrazol convulsions [5], the calcinosis elicited by vitamin-D compounds [6–8], digitoxin poisoning [9], the hypnotic action of pentobarbital and methyprylon [10], the adrenal necrosis produced by 7,12-dimethylbenz(a)anthracene [11], and the perforating jejunal ulcers with peritonitis elicited in the rat by indomethacin overdosage [12]. Probably many, if not all, of these protective effects are due to the induction by catatoxic steroids of hepatic microsomal drug-metabolizing enzymes [11, 12].

Adult male rats are more resistant than females to poisoning with picrotoxin but this sex difference is abolished by castration and absent in immature animals. Furthermore, testosterone raises the picrotoxin resistance in normal or spayed females but not in males [13]. Testosterone, like many other anabolic steroids, exhibits catatoxic potency when tested against various drugs, but this is true also of antimineralocorticoids which are not anabolic [3]. Hence, it seems of interest to determine whether picrotoxin poisoning can be prevented by a series of steroids previously shown to possess catatoxic actions against other drugs.

Methods

210 female Holtzman rats with an initial body weight of 100 g (range 90–110 g) were maintained exclusively on Purina Laboratory Chow and tap water, divided into 14 equal groups and treated as outlined in the Table. To obtain the best prophylactic effect, it is important to allow a few days of pretreatment with catatoxic steroids; hence, all animals received picrotoxin (B.D.H.) once, on the 4th day after initiation of steroid treatment, at the dose of 3.5 mg/kg subcutaneously in 0.2 ml of water.

The following steroids were tested: ethylestrenol (ORGANON), SC-11927 [that is, potassium 3-(3-oxo-9α-fluoro-11β, 17β-dihydroxy-4-androstene-17α-yl) (SEARLE)], spironolactone (SEARLE), norbolethone (WYETH), oxandrolone (SEARLE),

prednisolone acetate (ROUSSEL), progesterone (SCHERING), triamcinolone (LEDERLE), desoxycorticosterone acetate (CIBA), hydroxydione sodium hemisuccinate (PFIZER), cholesterol (MATHESON, COLEMAN and BELL) and β-sitosterol (S. K.F.). These steroids were administered by stomach tube at the dose of 10 mg in 1 ml of water twice daily; only in the case of triamcinolone did we have to reduce the dose to 2 mg since fatal intercurrent infections tend to develop with larger amounts of this highly potent glucocorticoid. One group of controls received 1 ml of water twice daily by a stomach tube (Group 1) and another 10 mg of sodium chloride in the same amount of water (Group 14).

The Table lists the mean degree of convulsions characteristic of picrotoxin poisoning in terms of an arbitrary scale in which 0 = no lesion, 1 = just detectable, 2 = moderate, 3 = most pronounced, as previously described [9]. However, for statistical evaluation we recognized only two grades: minor and sometimes dubious degrees of picrotoxin convulsions (between '0' and '+', in our scale) were rated as negative, all others as positive. These data as well as the mortality rates were then arranged in a 2×2 contingency table and the statistical significance computed by the Chi-square test. The experiment was terminated on the day after picrotoxin administration and the mortality rates are based upon the number of animals that died by that time.

Results

As shown in the Table, at the high dose employed here, picrotoxin produced virtually maximal functional disturbances and an 87% mortality among the absolute controls (Group 1). The convulsions were highly significantly inhibited by all the steroids that showed catatoxic effects against other drugs in our earlier work, namely ethylestrenol, SC-11927, spironolactone, norbolethone, oxandrolone, prednisolone and progesterone (Groups 2–8). On the other hand, the steroids which, in our previous studies, proved to be devoid of protective activity against other toxic substances (triamcinolone, desoxycorticosterone, hydroxydione, cholesterol and β-sitosterol) also failed to prevent picrotoxin convulsions (Groups 9–13). As expected, NaCl was likewise ineffective in this respect (Group 14). The mortality was highly significantly and, in most instances, totally prevented by the catatoxic steroids (Groups 2–8), but some decrease in mortality was also noted in several of the other groups, possibly as a consequence of cross resistance resulting from nonspecific irritation [14].

Protection by catatoxic steroids against picrotoxin intoxication.

Group	Treatment[1])	Convulsions[2])	Mortality[2]) (%)
1	H_2O	2.8	87
2	Ethylestrenol	0.8 ***	0 ***
3	SC-11927	1.1 ***	7 ***
4	Spironolactone	1.1 ***	13 ***
5	Norbolethone	0.9 ***	0 ***
6	Oxandrolone	1.5 ***	0 ***
7	Prednisolone acetate	1.2 ***	0 ***
8	Progesterone	0.8 ***	0 ***
9	Triamcinolone 2 mg	2.5 NS	40 *
10	Desoxycorticosterone acetate	2.2 NS	27 **
11	Hydroxydione sodium hemisuccinate	2.2 NS	40 *
12	Cholesterol	2.4 NS	27 **
13	β-Sitosterol	2.6 NS	73 NS
14	Sodium chloride	2.1 NS	33 **

[1]) In addition to the agents listed in this column, the rats of all groups were given picrotoxin as indicated in the text. The severity of the motor disturbances was estimated 30 min. after picrotoxin injection. Mortality was listed 24 hrs. later.

[2]) *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.005$, NS = Not significant.

Discussion

The most striking result of these investigations was that the steroids previously found to protect against many other types of intoxications are also most potent in inhibiting picrotoxin poisoning. Here, as in our earlier work, the catatoxic action was independent of the other pharmacologic properties of the steroids examined. Some of the active catatoxic compounds are anabolics (ethylestrenol, norbolethone, oxandrolone), others are antimineralocorticoids (SC-11927, spironolactone) virtually devoid of other properties. On the other hand, no significant anticonvulsive effect was associated with high mineralocorticoid (desoxycorticosterone), glucocorticoid (triamcinolone) or anesthetic (hydroxydione) potency. The hormonally inactive cholesterol and β-sitosterol were likewise devoid of anticonvulsive properties.

The most potent catatoxic steroids, e.g., spironolactone [15] and norbolethone [16], stimulate the proliferation of the smooth endoplasmic reticulum in hepatocytes, a feature allegedly characteristic of inducers of microsomal drug-metabolizing enzymes in general [17]. Furthermore, norbolethone and ethylestrenol, in doses in which they inhibit barbiturate anesthesia, increase the production of barbiturate-metabolizing enzymes, as shown by in vitro incubation of the hepatic microsomal fraction with this substrate [18]. Although there is no proof that all catatoxic actions depend upon the induction of drug-metabolizing enzymes in the hepatic microsomes, at least in several cases, this mechanism appears to be chiefly responsible for the protective effect. In any event, it would not be permissible to equate the catatoxic effect with hepatic enzyme-inducing ability since glucocorticoids are highly potent microsomal enzyme inducers; yet, only few of them exhibit any noteworthy catatoxic activity, and even this effect is limited to certain substrates. Presumably the various steroids activate different types of enzymatic activities, not all of which are equally effective in antagonizing the toxicity of different drugs. The mechanism of this differential effect, as well as the occasional dissociation between the anticonvulsive action of steroids and the protection against mortality induced by picrotoxin, will require further study.

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